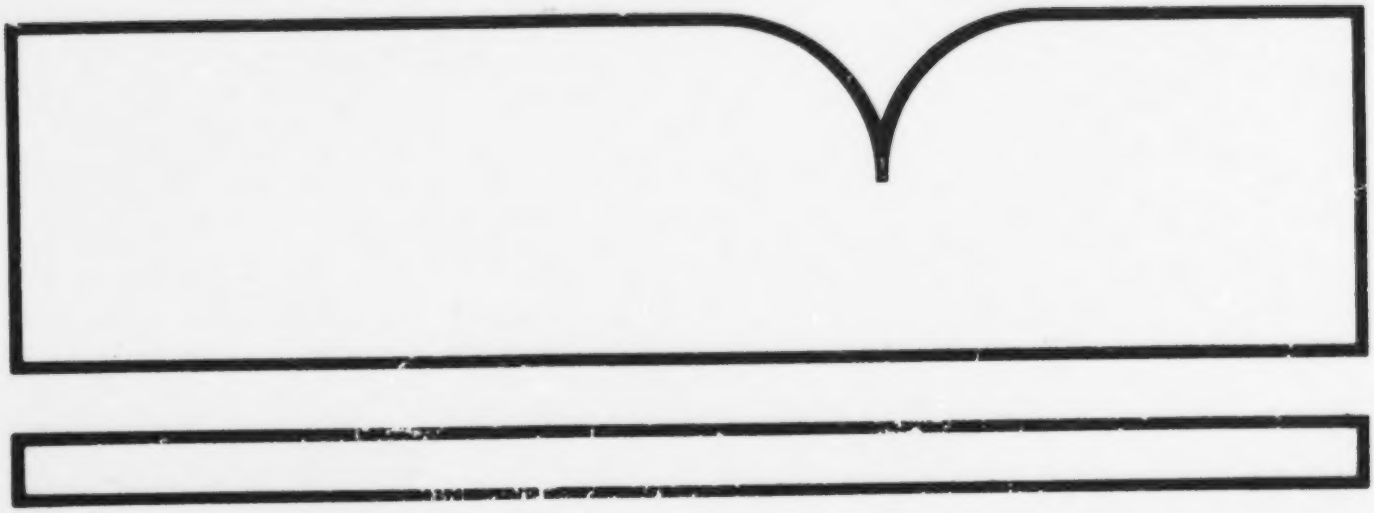
PB84-148782

Control of 'Giardia' Cysts by Filtration
The Laboratory's Role

(U.S.) Municipal Environmental Research Lab
Cincinnati, OH

Jan 84



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TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA-600/D-84-037		PB84 148782

4. TITLE AND SUBTITLE	5. REPORT DATE
Control of Giardia Cysts by Filtration: the Laboratory's Role	January 1984
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
Gary S. Logsdon, David W. Hendricks, Gordon R. Pyper	
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
USEPA, DWRD, Cincinnati, Ohio Colorado State U. Fort Collins, Colo. 80523 Dufresne-Henry, N. Springfield, Vermont 05150	BNC1A
	11. CONTRACT/GRANT NO. CR808650 CR809284
12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Municipal Environmental Research Laboratory--Cincinnati Office of Research and Development U.S. Environmental Protection Agency Cincinnati, Ohio 45268	seminar proceedings
	14. SPONSORING AGENCY CODE EPA/600/14

15. SUPPLEMENTARY NOTES

16. ABSTRACT

USEPA has sponsored research on the ability of slow sand, diatomaceous earth, and dual or multi-media filters to remove turbidity causing particles, coliforms, and Giardia cysts. The high volume Giardia sampler used during disease outbreaks was not appropriate for this research. Membrane filters with diameters of 142 mm or 293 mm and 5 um pore size were used to concentrate cysts from filtered waters. This technique has been used to evaluste cyst removal capabilities of the above-mentioned types of pilot filters and of small system sized (10 to 30 gpm) granular media, slow sand and diatomite filters.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	27
	20. SECURITY CLASS *(This page)* UNCLASSIFIED	22. PRICE

EPA Form 2220-1 (9-73)

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EPA Cooperative Agreement Numbers
CR808650
CR809284

MUNICIPAL ENVIRONMENTAL RESEARCH LABORATORY
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INTRODUCTION

Dramatic improvements have been made in the quality of drinking water in the USA since the turn of the century. Waterborne epidemics of cholera and typhoid fever are rare events now. Nevertheless, the need for vigilence continues, as in recent decades we have become aware that other biological agents are causing waterborne disease.

One notable example is giardiasis, a disease that received very little attention before the 1960's. According to Rendtorff (1), "*Giardia* was thought in the 1950's to cause occasional problems of diarrhea in children but its appearance was so common and, in adults so lacking in clinical symptomology, that most considered it a non-pathogen." Recently Craun and McCabe (2) reported that for the 1971-1979 time period, giardiasis was the cause of 11 percent of the waterborne disease outbreaks in the USA. Waterborne giardiasis outbreaks have continued to occur in the 1980's. The need to improve water treatment practice continues in some locations, as proven by the giardiasis outbreaks.

For several years, the Drinking Water Research Division has been conducting and sponsoring research on water filtration in order to develop information needed by water utilities that treat waters that might be contaminated by *Giardia* cysts. This paper presents information on some of the laboratory methods used in this work and briefly relates preliminary findngs of recent research.

CYST SOURCES

One of the significant obstacles to conducting research on water filtration for Giardia cyst removal has been and continues to be the lack of a readily available and easily maintained source of the cysts. Giardia cysts can not be cultured and grown, as can E. coli, for example, so they must be obtained from warm blooded animals that are infected and shedding cysts. Donors have included mice, dogs, and humans.

In early cyst removal research performed by the Drinking Water Research Division (DWRD), cyst models (9 um radioactive beads) and G. muris cysts from mice were used (3). Giardiasis is not a common disease in Cincinnati, where the DWRD laboratory is located, so obtaining cysts from human donors has been very difficult. In order to obtain research results in a timely fashion, DWRD performed exploratory studies with cysts other than G. lamblia.

Studies later funded by DWRD have used Giardia cysts from human donors or from dogs. Granular media filtration research at the University of Washington (4) was done with G. lamblia from humans. The quantity of cysts was limited, and the supply varied from time to time; thus much of the work was based on counting of cyst-sized (8 to 12 um) particles.

Research at Colorado State University (CSU) has been conducted with Giardia cysts obtained from dogs. Cysts from dogs have been reported to have caused human infection (5), and G. lamblia cysts from human stool specimens and G. lamblia trophozoites (Portland-1 strain) have caused infections in dogs (6). The physical characteristics of Giardia cysts from humans and from dogs seem to be very similar or identical. For purposes of water filtration studies, use of Giardia cysts from dogs should be as appropriate as use of Giardia cysts from human donors.

Filtration research at McIndoe Falls, Vermont has been conducted with cysts from human donors. G. lamblia cysts were obtained from stool samples through the cooperation of the Vermont Health Department and the Mary Hitchcock Hospital in Hanover, New Hampshire.

CYST DOSING AND SAMPLING PROCEDURES

CSU Dosing

To obtain cysts for pilot scale filtration experiments conducted at the Environmental Research Center (ERC) at Colorado State University, cysts were separated from canine fecal samples by mixing the feces in water, allowing the sediment to settle, and saving the supernatant (7). If the sample was quite dirty it was filtered through cheesecloth. The processed cyst samples were refrigerated until use.

When a filtration experiment was to begin, cysts were added to a temperature controlled milk cooler filled with Horsetooth Reservoir water and kept at 5° or 15° C. The cyst concentrations in the feces suspensions were determined

by three to five analyses of each suspension prior to adding it to the milk cooler. The concentration of cysts in the milk cooler was then calculated, and designated as the "concentration added." The cyst concentration in the milk cooler was determined also by analyzing a sample from the cooler. This sample was obtained by pumping water from the milk cooler through the polycarbonate membrane sampling system, and the measured cyst concentration was designated as the influent "detected" Giardia concentration.

The size of the water sample taken from the milk cooler ranged from 2 to 15 L. The sample volume was limited by the amount of water that could be filtered through the membrane filter before a pressure of 10 psi was reached. A 200 mL grab sample was also collected from the cooler and used for standard plate count and coliform analyses.

For field testing at CSU using a 1 gpm diatomaceous earth filter, cysts were added to a 700 L raw water feed tank. Raw water was sampled by the membrane filter method to determine the concentration of cysts in the raw water. When the 20 gpm mobile rapid sand pilot plant was used, cysts were dosed on a continuous basis from a suspension of known concentration. The cysts were added to the raw water and mixed before treatment chemicals were added.

Vermont Dosing

In the Vermont research, cysts were stored in a 5% formalin solution at 0° to 4° C until processed for concentration by sucrose separation. The concentrate after the separation step was stored at 4° C until used.

In the research at McIndoe Falls, cyst concentrates were counted at the University of Vermont. Three subsamples were used and counted with a hemacytometer to determine the number of cysts. The cyst concentrate was then shipped on ice to McIndoe Falls. Because the filters were operated at 10 to 15 gpm flows (characteristic of very small systems) the cysts were added as an instantaneous slug, in order to present the maximum challenge to the filters.

CSU Sampling

Polycarbonate membrane filters were used to sample for Giardia cysts at CSU for both influent feed tank water and filter effluent. For most experiments, filters having 142 mm diameter and 5 um pores were used. Larger filters (293 mm) were used to sample the effluent from the 20 gpm mobile filtration plant.

Sampling protocol for the feed tank was described by Silverman (8): The filter holder was washed and the membrane was installed, tap water was introduced into the filter device from the effluent side, the filter was then attached to the sampling pump, the remaining air was bled off, and water was pumped through the filter. This was done very carefully in order to avoid creating pressure disturbances when filter effluent samples were taken. The volume of water passed through the membrane was measured and recorded so that the cyst concentration could be calculated. When sampling was complete, the flow of water to the membrane filter was shut off, excess water in the filter chamber was removed by vacuum, and the apparatus was opened. The top and bottom of the filter holder and the membrane were

all thoroughly washed into a Pyrex® dish to recover cysts. The washings were sealed in a mason jar, labeled, and refrigerated until they could be concentrated and counted. Sampling protocol for the effluent side was identical, except the pump was not needed for pilot filters operating under pressure, which included the laboratory scale rapid sand filter, three slow sand filters, and the DE filter. Evaluation of the membrane sampling procedure gave 45% average recovery, with a range of 36% to 54%.

Vermont Sampling

Sampling of the filter effluent for cysts at Vermont consisted of pumping water at 1 gpm (about 8% of sand filter effluent) from the test filters to a membrane filter holder equipped with a water meter and pressure gauge. Polycarbonate membrane filters having a 293 mm diameter and 5 um pores were used. The meter reading was recorded and timed at the start and at the end of the sampling period in order to determine the volume of water sampled for cysts. Sampling was continued for 24 hours per sample, in the slow sand filter runs, and pressure on the influent side of the filter never exceeded 10 psi.

When sampling was completed, all the water in the filter holder was drained by an aspirator. The filter membrane was removed from its holder by means of tweezers, folded to the center to retain any moisture, and placed in a one quart wide mouth mason jar. Adding 50 mL of distilled water to the jar containing the filter membrane was found to be advantageous when reduction of interference from background material was needed. Formalin-fixed cysts did not disintegrate, but some naturally occurring biological materials did. The jars were tightly sealed and shipped in an iced condition to the laboratory for counting.

The cyst sampling methods used in these two research projects differed from the sampling technique described in Standard Methods (9). The method described in that publication has been used to sample raw and finished waters during outbreak investigations. Previous filtration studies (3, 4) used membrane filtration for cyst sampling because small (1 liter) and medium volumes (about 100 liters) could be sampled by membrane filters, in contrast to the 1900 L volume recommended for the yarn-wound orlon filters. Thus small pilot filters could be used and the volume of filtered water produced would be sufficient for analysis. Membrane filters have continued to be used for research on filtration for Giardia cyst removal even when larger systems are tested. This provides some continuity in procedures and is helpful when comparisons are made between various studies. Another factor favoring use of membrane filters for these studies is the high variability of cyst recovery inherent in the orlon yarn-wound filter sampler. Cyst recovery by membrane filter is higher and less variable, by comparison.

CYST COUNTING METHODS

CSU Counting

At CSU the cyst enumeration procedures were done under direction of Dr. C. P. Hibler at the Pathology Laboratory, and depended on the nature of the sample. The zinc sulfate flotation technique was used in 1981 at the

start of the project. After investigation of other techniques the micropipette technique was adopted in July, 1982, as the latter method was found to be more accurate.

Fecal samples of cyst suspensions were analyzed according to the estimated concentration of cysts present. If the initial zinc flotation procedure indicated a relatively large number of cysts in the fecal sample, the actual number of cysts in the sample could be determined by the Stoll dilution technique. Samples having low cyst concentrations were less desirable and had to be concentrated and then counted by either the zinc flotation or the micropipette method.

The Stoll dilution technique consisted of adding 3 mL of Lugol's Iodine to a Stoll flask which was then filled to the 56 mL mark with cool distilled water. Four mL of thoroughly mixed fecal suspension were placed in the Stoll flask, and the flask was shaken to mix the contents. A 0.075 mL aliquot was removed from the flask via micropipette and placed in a vaseline well on a glass slide. A coverslip was affixed to the slide, and the slide was examined for Giardia cysts at 400 x magnification. The total number of cysts seen on one slide was multiplied by 200 to give the total number per mL in the sample. A minimum of 2 slides was prepared as described, read, and averaged.

Cyst suspension containers storing processed cysts ready for filtration experiments were labelled with the date and the number of cysts per mL. The samples were counted at least every third day, or before a portion was used, to ascertain that the cysts had not begun to degrade.

Samples obtained during operation of the pilot filters were processed by either the zinc flotation method or the micropipette technique. In the zinc flotation method, a fecal sample the size of a pea was placed in a centrifuge tube, 5 to 6 drops of Lugol's Iodine were added, and the sample was mixed well. The tube was filled half way with zinc sulfate ($ZnSO_4$, S.G. = 1.18) solution and mixed. The tube was then filled with zinc sulfate solution until the meniscus bulged at the top. A coverslip was affixed to the tube, and the coverslip was tapped with the end of a pencil to firm a secure bond. Next the tube was centrifuged for 5 minutes at 1500 rpm. The coverslip was removed from the tube, placed on a glass slide, and examined for Giardia cysts at 100 x magnification.

Samples obtained by the membrane filter sampling technique were brought to the CSU Pathology Laboratory and stored in the refrigerator overnight to settle the cysts and debris. The following day the samples were reduced in volume by pipette to less than 50 mL without disturbing the sediment that had formed. This remaining volume was remixed and poured into a 50 mL conical centrifuge tube. The tube was centrifuged for 5 minutes at 1500 rpm. The supernatant was then pipetted off until about 5 mL remained, and the procedure was repeated until finally all the sample had been concentrated to 1 mL and the sample jar had been rinsed well with distilled water.

Once the sample had been concentrated to 1 mL volume, either of the two counting methods were used to determine the cyst concentration. The first method used was zinc flotation. Later, the micropipette technique was used. This method entailed adding 5 to 6 drops of Lugol's Iodine

to the 1 mL concentrated sample, mixing thoroughly, and withdrawing an aliquot of 0.05 mL. This volume was then placed in a vaseline well on a glass slide, a coverslip was affixed to the slide, and the slide was examined at 400 x magnification. If cysts were seen, a minimum of two aliquots were counted and averaged. The total number of cysts in the sample was found by multiplying by a factor of 20. Counts of replicate samples counted by micropipette varied from the mean by ± 20%.

Vermont Counting

In the McIndoe Falls study, cyst enumeration procedures were done under the direction of Dr. Robert Sjogren at the University of Vermont. Polycarbonate membrane filters were transported on ice from the field in one quart wide mouth glass mason jars. Jars and folded membrane filters were stored at 0° to 4° C with distilled water or 5% buffered formaldehyde at the University of Vermont.

Filters were processed by cutting them into 5 cm pieces and placing the pieces in a 40 oz. glass Waring blender cup. Any excess fluid in the sample jar was poured into the blender cup, and the jar was washed twice with about 20 mL of distilled water. This was added to the blender cup, and the volume of fluid for blending was made up to 200 mL. A few drops of liquid dishwashing detergent (Ivory®) previously diluted to 1:100 were added. The cut membrane was blended for 30 seconds at 20,000 rpm using a single speed blender base.

The contents of the blender were strained through two thin layers of cheese cloth held in a large glass funnel supported by a 1 L glass beaker. The blender cup was washed two or three times with about 30 mL of distilled water from a spray bottle. The cut pieces of membrane were stirred briefly with a wooden applicator stick during this process. The cheese cloth was then carefully squeezed to remove excess water. All fluid was collected in the 1 L beaker.

After the filtrate was collected, it was distributed among several 50 mL glass conical centrifuge tubes which were spun at 450 rpm for 15 minutes using a swinging bucket rotor centrifuge. The supernatant was then carefully aspirated from each tube, leaving the undisturbed pellet in the bottom along with about 2 mL of liquid. The pellets were again suspended and the contents of the 50 mL tubes were combined in a 15 mL conical glass centrifuge tube. All of the 50 mL tubes were rinsed in serial fashion with distilled water that was also added to the 15 mL tube. The Pastuer pipette used to transfer material was also rinsed and that liquid was added to the 15 mL tube. The 15 mL tube was then centrifuged in a clinical table top centrifuge for 15 minutes at 450 rpm.

Upon completion of the second centrifugation, the supernatant in the 15 mL tube was carefully removed using a Pastuer pipette until a final volume of 0.3 mL of pellet and fluid was left. To this was added 0.1 mL of fresh 5% Lugol's Iodine solution. The tube contents were mixed, and a portion of the cyst suspension was examined using a counting chamber and binocular microscope. Experiments demonstrated that a volume of 0.4 mL gave the best counting reproducibility.

Laboratory tests were performed to evaluate several aspects of the University of Vermont procedure. The blender method using detergent gave satisfactory results in determinations of cyst recovery when diluted suspensions were prepared as a method check. A study to determine if blending destroyed cysts revealed that the 30 seconds of blending did not result in loss of cysts. Four separate experiments were conducted to determine average cyst recovery from the 293 mm membrane filters. Recovery ranged from 50% to 60%. Formalin treated cysts, when stored in distilled water, did not decrease in numbers or show signs of deterioration during two months of storage. Of several different treatments of the cysts after they were in pellet form, staining with Lugol's Iodine was preferred, although use of acridine orange showed some promise for easier cyst identification.

Laboratory methods for other water quality parameters of interest in this study have been developed and standardized for a number of years. Coliform measurements in the CSU and McIndoe Falls studies were done according to Standard Methods (9). Analyses were performed at CSU and at Spectrum Environmental Research Laboratory in Montpelier, Vermont. Samples collected at sites away from the analytical laboratories were shipped on ice to the laboratories. Turbidity was measured on site in both projects with bench top turbidimeters or flow through turbidimeters or both.

FILTRATION EQUIPMENT USED

Filtration research at CSU has been conducted with pilot scale rapid rate granular media filters, slow sand filters, and a diatomaceous earth (DE) filter. Full scale, small system sized filters were also tested in these research projects. CSU used a 10 to 30 gpm rapid rate granular media package filtration plant. A 13 gpm slow sand filter and a similarly sized DE filter have been studied at McIndoe Falls.

CSU Pilot Filters

The CSU pilot scale rapid rate filters were operated in an "in-line" mode, using two stage rapid mixing followed by filtration (10). Rate of flow was from 0.4 to 0.7 L/min, and filtration rates were in the 5 m/hr to 12.5 m/hr range. Filter columns of 5 and 10 cm diameter were used. Sand filters had 76 cm of 0.45 mm e.s. (effective size) sand with a uniformity coefficient (u.c.) of 1.5. The dual media filters had 46 cm of 0.9 mm e.s. anthracite (u.c. = 1.7) over 30 cm of 0.45 mm e.s. sand (u.c. = 1.5). Coagulation was accomplished with alum or polymer or with combinations of alum and polymer. Some filter runs were made with uncoagulated water to show the detrimental effects of failing to coagulate clear waters before they are filtered.

Three pilot scale slow sand filters were assembled at CSU for studies of this process. The filters, described by Bellamy et al. (11), were 30 cm diameter columns filled with 96 cm of 0.28 mm e.s. sand (u.c. = 1.5) supported by 46 cm of coarse sand and gravel. The filters operated at rates of 0.04, 0.12, and 0.40 m/hr (1, 3, and 10 million gallons per acre per day, or mgad) with raw water fed from a common tank so the effects of

filtration rate could be evaluated. No coagulation chemicals were used, because water generally is not coagulated before it is applied to slow sand filters. Water temperatures for these experiments were approximately 5° C or 15° C. The three filters were operated to conventional slow sand filter head losses (about one meter) but because of limited ceiling height they were operated in a pressure filter mode. Later seven more filter columns were constructed. These have been operated in a gravity mode because they were located in facilities with adequate head room for columns 3.3 meters high. The seven columns included some with different sand sizes and different media depths, so other design variables could be evaluated.

The diatomaceous earth pilot filter used at CSU was a 0.093 m^2 DE filter similar to the one described by Logsdon et al. (3). The filter was operated at filtration rates ranging from 2.4 to 9.8 m/hr (4 to 15 L/min or 1 to 4 gpm flow rate). Operation of the DE filter consisted of precoating, filtration, and backwashing. DE grades used for precoating and for body feed included Celite 545®, Celite 535®, Celite 503®, and Hyflo Super Cel®. Although normal DE filtration practice is to terminate a run because terminal head loss has been reached, in this research most of the runs were terminated because of operating time considerations rather than because terminal head loss had been reached.

CSU Small System Filter

The small scale granular media filter used at CSU was a Neptune-Microfloc WB-27 package plant that previously had been mounted on a trailer so it could be transported to locations where water filtration studies were to be carried out. It had been used earlier at Leavenworth and Hoquiam, Washington (4) and at Canton and Oneida, New York (12). This plant has a filter area of 0.37 m^2 (4 ft^2) and can operate in the 38 to 114 L/min (10 to 30 gpm) flow range. It was operated in an in-line or direct filtration mode by CSU.

Vermont Small System Filters

The slow sand filter at McIndoe Falls was one of two filter beds in a plant built in 1976 when the springs that had previously served as the community's water source were replaced. Later, ground water was developed by the community, so the slow sand filter was removed from service. Because it was not being used to produce potable water, it could be used for EPA-sponsored research. The filter bed used in this study was 5.9 x 6.7 m (19.5 x 22 ft). Flow at 51 L/min (13.6 gpm) resulted in a rate of filtration of 0.08 m/hr (2 mgad). The filter was designed to have 1.07 m (42 in) of 0.33 mm e.s. sand (u.c. = 2.7).

The DE filters used during the McIndoe Falls research were pressure filters. The first set of experiments was performed with a rented unit that had a septum area of 0.74 m^2 (8 ft^2). Later tests were made with a filter having a septum area of 0.93 m^2 (10 ft^2). Both units used slurry pumps to add body feed to the raw water. The grade of DE studied at McIndoe Falls was Celite 503®.

PILOT SCALE RESULTS

CSU Research Results

Preliminary results of rapid rate granular media filtration studies have been presented by Al-Ani and Hendricks (10). They reported that coliform removal by filtration (without disinfection) could be as high as 99.9% or more, when raw water spiked with sewage was filtered after being adequately coagulated. Previously presented results plus more recent data show that high removals of turbidity relate well with coliform removal when clear surface waters of less than 1 NTU are coagulated and filtered. Table 1 shows that when filtered water turbidities were 0.10 NTU or lower coliform counts were equal to or less than 1 per 100 mL and coliform removal efficiencies met or exceed 99.0% for 70% of all samples taken.

Granular media filtration research results from CSU also showed that Giardia cysts could be removed effectively when properly coagulated water was filtered. Cyst removal percentage exceeded 99% in numerous filter runs. The relationship between cyst breakthrough and turbidity in coagulated, filtered water is shown in Table 2. The frequency of occurrence of effluent samples having 5 or fewer cysts per liter decreased as filtered water turbidity increased. The same trend was seen for samples showing cyst removals of 99% or greater. A similar trend could be seen in the data analyzed if the occurrence of finding no cysts in the filtered water had been reported. The detection limit varied from run to run, however, depending on the volume of filtered water passed through the membrane filter sampling device, so those results would be more difficult to interpret.

The effect of using no chemical pretreatment is shown clearly in Table 3. Percent removals of Giardia cysts ranged from 36% to >99.99%, while percent removals of total coliform bacteria were -7% to 99.9%, and turbidity decreased as much as 77% in some runs but increased up to 72% in others. These results contrast sharply with removals of cysts and total coliforms when effective chemical pretreatment is used. The message is clear: effective chemical pretreatment is imperative if cysts and bacteria are to be removed adequately. It is clear also that if turbidity removal is 80% or greater, when raw water turbidity is less than about 1 NTU, removals of total coliform bacteria and Giardia cysts will exceed 99%.

Results of the CSU slow sand filtration studies, shown in Table 4, indicate that the filters were able to remove a variety of microorganisms. Total coliform removal ranged from >99.99% to >99.29%. Standard plate count removals were lower, ranging from 92.7% to 95.8%. Fecal coliform removal ranged from >98.4% to >99.8%. Removals were lowest at the highest filtration rates for all three bacterial parameters studied. An interesting aspect of this research was the lack of a relationship between removal of biological particles (total coliform, standard plate count, and fecal coliform) and removal of turbidity when Horsetooth Reservoir water was the raw water source. Average filtered water turbidity from all filters exceeded 1 NTU, ranging from 3.5 to 4.1 NTU. Raw water turbidity ranged

TABLE 1

INFLUENCE OF FILTERED WATER TURBIDITY ON COLIFORM REMOVAL BY COAGULATION AND FILTRATION, CSU

Filtered Water Turbidity Range NTU	Number of Samples	Percent of Samples with	
		Coliform ≤ 1/100 mL	Coliform Removal ≥ 99.0 %
≤ 0.10	42	69%	71%
0.11 to 1.4	46	37%	50%
> 1.4	19	47%	42%

TABLE 2

INFLUENCE OF FILTERED WATER TURBIDITY ON GIARDIA CYST REMOVAL BY COAGULATION AND FILTRATION, CSU

Filtered Water Turbidity Range NTU	Number of Samples	Percent of Samples with	
		≤ 5 cysts/L	Cyst Removal ≥ 99.0%
< 0.10	23	78%	83%
0.11 to 1.4	28	36%	43%
> 1.4	3	33%	33%

TABLE 3

CSU RAPID RATE FILTRATION RESULTS -- NO CHEMICAL PRETREATMENT

Filtration Rate m/hr	Turbidity NTU			Total Coliform Number per 100 mL			Giardia Cysts Cysts/L		
	Infl.	Effl.	% Removal	Infl.	Effl.	% Removal	Infl.	Effl.	% Removal
5.1	0.22	0.16	27%	4000	3450	14%	260	66	74%
13.6	0.22	0.38	- 72%	4000	3000	25%	260	9	96%
5.0	0.22	0.26	- 18%	1300	800	38%	458	0.6	> 99.8%
13.3	0.29	0.33	- 14%	1300	520	60%	458	0.01	> 99.99%
4.9	1.7	1.77	- 1%	20,000	2000	90%	754	58	92%
4.9	1.7	1.50	12%	20,000	2000	90%	754	184	76%
12.9	1.7	1.18	31%	20,000	2000	90%	754	86	87%
12.9	1.7	1.69	1%	20,000	2000	90%	754	83	89%
4.9	2.4	1.59	34%	--	--	--	1026	0.9	99.9%
5.0	3.2	2.40	20%	59,000	50	99.9%	1012	1.1	99.9%
4.9	3.2	0.73	77%	--	--	--	975	0.5	99.9%
24.8	1.6	1.3	19%	4650	4400	5%	269	156	42%
19.2	1.6	1.3	19%	4650	5000	- 7%	269	172	36%
12.4	1.6	1.31	19%	4650	4600	1%	269	172	36%
5.8	1.6	1.35	16%	4650	< 1	99.9%	269	85	68%
24.2	1.6	1.19	26%	200	200	0%	245	125	49%
19.6	1.6	1.23	23%	200	200	0%	245	68	72%

TABLE 4

AVERAGE BACTERIA REMOVAL BY SLOW SAND FILTRATION, CSU

Filtration Rate m/hr	Total Coliform Removal	Standard Plate Count Organism Removal	Fecal Coliform Removal
0.04	>99.99%	95.5%	>99.82%
0.12	>99.95%	95.8%	>98.43%
0.40	>99.29%	92.7%	>98.63%

from 5 to 10 NTU. The failure of the slow sand filters to produce filtered water meeting the 1 NTU MCL was not anticipated. When the filtered water turbidity exceeded expected values, samples of raw water were subjected to additional testing to determine the size characteristics of the particles. The results of turbidity analysis after 5.6 NTU water was passed through various sizes of membrane filters ranging from 0.22 to 8 um indicate that 64% of the turbidity remained after filtration through the 1.2 um membrane and 27% remained after filtration through the 0.45 um membrane. Later the fine particles passing through the slow sand filter were reported to be kaolinite and montmorillonite clay particles.

The capability of slow sand filters to remove Giardia cysts was demonstrated in the CSU research. Results from Bellamy et al. (11) have been analyzed and are presented in Table 5. Cyst removal is influenced strongly by filter operating conditions. Removal of cysts was least effective when a new filter was placed in operation, before a biological population (a variety of organisms including protozoa, rotifers, and bacteria) could become established in the filter sand and in the coarse sand and gravel support media.

When the slow sand filters had a well developed biological population (minimum sand bed exposure was 29 weeks), Giardia cyst removals were nearly 100 percent, even though in some cases the filter had been scraped and the age of the schmutzdecke was as low as three weeks. The lowest percentages of cyst removal attained with the developed sand bed was 99.77%, and cyst removal was 99.90% or greater in 22 of 24 observations. In 12 of 24 observations, cysts were not found when filtered water volumes ranging from 81 L to 1,440 L were passed through 5 um pore diameter membrane filters to test for presence of Giardia cysts.

An evaluation of more severe operating circumstances showed that cyst passage could occur when no schmutzdecke was present on the sand surface and when new sand and gravel support media had been installed in the slow sand filter. Slow sand filtration often was effective for Giardia cyst removal, even in the absence of a schmutzdecke, if the filter bed contained an established biological population. This suggests that Giardia cyst removal occurs not only in the schmutzdecke, but also within the depth of the sand bed.

Research performed at CSU with the diatomaceous earth test filter has confirmed the earlier observation of Hunter, et al. (13) showing that fewer total coliforms passed through the DE filter as finer grades of diatomite were used. The CSU results, showing that removals of standard plate count organisms, total coliforms, and turbidity all improve as finer grades of DE are used, are presented in Table 6. Because of the size differences of bacteria vs. the fine clays in Horsetooth water, the removal of turbidity-causing particles was less than the removal of

TABLE 5

EFFECT OF SLOW SAND FILTER CONDITIONS ON REMOVAL OF *GIARDIA* CYSTS, CSU

Filter Media Condition	Filtration Rate, m/hr	No. of Samples	Effluent Concentration Range, cysts/liter	No. Samples with NO Cysts Detected	Cyst Removal Percentage Range
New Media, no established biopupulation	0.40	1	17	0	99.1
New sand, old support media	0.12	1	<0.04	1	99.9
No schmutzdecke, but sand has established biological population	0.04	6	<0.04 to 5.4	4	98.7 to 99.9
	0.12	5	<0.01 to 1.5	4	99.6 to 99.9
	0.40	4	<0.04	3	99.9
Both schumtzdecke and biological population established in sand	0.04	8	<0.06 to <1.0	5	99.7 to 99.9
	0.12	8	0.04 to <0.4	4	99.9
	0.40	8	0.01 to 1.4	3	98.8 to 99.9

TABLE 6

AVERAGE REMOVAL PERCENTAGES OF TOTAL COLIFORM BACTERIA, STANDARD PLATE COUNT BACTERIA, TURBIDITY AND *GIARDIA* CYSTS WITH VARIOUS GRADES OF DIATOMACEOUS EARTH, CSU

Grade of Diatomaceous Earth	Celite 545®	Celite 535®	Celite 503®	Hyflo Super-Cel®	Celite 512®	Standard Super-Cel®	Filter Cel®
Number of tests	15	2	10	4	2	3	2
Median Particle Size (um)	26.0	25.0	23.0	18.0	15.0	14.0	7.5
Total Coliform Removal (%)	49.46	85.45	69.41	92.60	97.95	99.90	99.86
Standard Plate Count Removal (%)	58.42	80.51	69.10	75.29	78.63	99.11	99.85
Turbidity Removal (%)	13.36	12.50	11.97	23.56	31.14	49.77	97.64
Giardia Cyst Removal (%)	>99.7	>99.5	>99.7	>99.4	--	--	--

microorganisms for most grades of DE. The finest grade tested, Filter Cel®, was very effective for turbidity removal. This grade usually is not used in potable water treatment because of the high head loss caused by small particle size.

A comparison of turbidity removal by diatomaceous earth filtration and membrane filtration was made (7). A 5 um filter permitted passage of about 98% of the turbidity, more than the 88% passed by Celite 503®. A 1.2 um filter passed 64%, similar to the 72% passed by Celite 512®. The 9% passed by a 0.2 um membrane filter was similar to the 2% passed by FilterCel®. These results indicate that the particle sizes passed by various grades of DE may be estimated by a comparative analysis of the effluent turbidity from membrane filters and DE filters.

Research with alum-coated DE showed that diatomite onto which aluminum hydroxide had been precipitated was more effective than uncoated diatomite for removal of standard plate count organisms, total coliforms, and turbidity. With Celite 545® turbidity removal improved from 13% to 98% and total coliform removal increased from 49% to 99.86%. Alum coating enabled the diatomite to remove smaller particles than uncoated DE could remove, The results suggest that filtered water turbidity might be more closely related to the microbiological quality of DE filter effluent when the alum coating process is used.

Field tests of DE filtration were also conducted using two mountain stream sources. A one square foot test filter was used at these sites at filtration rates of 2.4, 4.9, and 9.8 m/hr. Temperatures ranged from 3.5 to 10°C, and raw water turbidities varied from 0.55 NTU to 32 NTU. In these field tests a coarse grade of diatomite, Celite 545®, was used. Coliform removals were less effective at the 4.9 and 9.8 m/hr filtration rates than at the commonly used rate of 2.4 m/hr (42% and 57% at higher rates vs 51% to 97% obtained at 2.4 m/hr). Turbidity percent removal was much higher on these waters than on Horsetooth Reservoir water. The highest turbidity removal with the latter water was 24% when Celite 545® was used, and the average removal for all Celite 545® runs on that water was 13%. In contrast, with the other raw waters, turbidity removal ranged from 21 to 77%. Turbidity removal by diatomaceous earth filtration is in part a function of the nature of the water being treated.

Giardia cyst removal was studied with the DE test filter and Horsetooth Reservoir water. Of the 26 runs, the only one in which cysts were detected had a very high influent cyst concentration, 34,000 cysts per liter. When influent cyst concentrations ranged from 100 to 10,000 cysts/L, no cysts were detected in the filtered water. Results in Table 7 show that DE filtration is effective for cyst removal at rates of 2.4 to 9.8 m/hr. These experiments were performed with a raw water source having very fine turbidity-causing particles. The data in Table 7 very clearly show that cyst removal efficiency was not related to turbidity removal by DE filters.

TABLE 7

GIARDIA CYST AND TURBIDITY REMOVAL BY DE FILTRATION, CSU RESULTS

Celite® Grade	Number of Runs	Filt. Rate m/hr	Giardia Cyst Concentration Added to Raw Water - cyst/L	Giardia Cyst Concentration In Filtered Water - cyst/L	Number of Cysts Found in Filtered Water Sample	Cyst Removal Percentage
545	4	2.4	100 to 770	<0.3 to <0.7	None	>99.3 to >99.95
545	4	2.4	2470 to 10,000	<0.0004 to <0.11	None	>99.998 to >99.999
545	1	2.4	3.36×10^4	25	1700	99.92
545	3	4.9	100 to 500	<0.3 to <0.4	None	>99.5 to >99.93
545	1	4.9	8850	<0.4	None	>99.995
545	2	9.8	100 to 500	<0.4 to <3.3	None	>99.3 to >99.5
535	1	2.4	100	<0.4	None	>99.5
535	1	4.9	100	<0.4	None	>99.5
503	2	2.4	100 to 500	<0.3 to <0.7	None	>99.7 to >99.8
503	1	2.4	5500	<0.06	None	>99.998
503	3	4.9	100 to 500	<0.4 to <0.9	None	>99.5 to >99.8
503	1	9.8	100	<0.5	None	>99.4
Hyflo	1	2.4	100	<0.5	None	>99.5
Hyflo	1	4.9	100	<0.7	None	>99.3

Field tests for removal of Giardia cysts were performed at the Cache la Poudre River and at Dillon, Colorado, at filtration rates of 2.4 to 9.8 m/hr. Celite 545® was used in the six filter runs in which cyst removal was studied. In five of the six runs, no cysts were detected, and removals exceeded 99.93% to 99.99%, depending on influent cyst concentration and the volume of filtered water sampled for cysts. Filtered water turbidity in these runs ranged from 0.3 NTU to 1.5 NTU.

During one of the six filter runs, cysts passed through the filter. When the filter was then subjected to leak testing, existence of a leak was confirmed. The turbidity of the filtered water in the run in which the leak occurred was 7.3 NTU, and cyst removal was 99.4%. These results suggest that when DE filtration equipment is properly maintained, this process is capable of removing over 99.9% of Giardia cysts from raw water, and that process efficiency is related to proper operation and maintenance of equipment rather than to effluent turbidity.

SMALL SCALE SYSTEM RESULTS

CSU Rapid Rate Granular Media Filtration Results

The trailer mounted Water Boy® filtration plant was used to corroborate results found using the laboratory scale pilot plant. Preliminary testing without chemicals was done during the summer of 1983 using raw water from Horsetooth Reservoir. The results for Giardia cysts, total coliform bacteria and turbidity show percent removals of about 50%, 30%, and 15%, respectively. These results are virtually the same as those obtained with the laboratory scale pilot filters when they were operated without chemical pretreatment. Removals of Giardia cysts, total coliform bacteria, and turbidity were about 99.9%, 99.9/%, and 95% respectively when effective chemical pretreatment was used, e.g. sufficient to remove 80% of the influent turbidity or to produce a filtered water turbidity below 1.0 NTU, whichever gave the lower turbidity. Again, these results were virtually the same as those obtained with the laboratory scale rapid sand filtration plant. More testing is presently under way with Cache La Poudre River water as the source water for conditions of low turbidity, e.g. below 1 NTU.

McIndoe Falls, Vermont Slow Sand Filter and DE Filter Results

One of the two filter beds of the McIndoe Falls slow sand filter has been operated for research purposes most of the time since May, 1982. Ambient raw water quality data for the first eleven months of the study are shown in Table 8. The brook that serves as a raw water source drains a marsh that would probably have numerous animals, thus the coliform counts were higher than expected.

The slow sand filter's capability for removing bacteria and turbidity is shown in Table 9. Under ambient water quality conditions, bacteria removal was slightly better during times of warm water than it was when the water was cold. This is expected, because biological processes

TABLE 8

RAW WATER QUALITY AT McINDOE FALLS FROM 5/10/82 TO 4/8/83

	Number of Samples	Maximum	Minimum	Average
Standard Plate Count, per 1 mL	91	5100	0.5	35
Total Coliform, per 100 mL	91	3000	7	350
Turbidity, NTU	340	59	0.3	1.0
Temperature, °C *	340	25	0	10

*5°C or below 45% of time
Above 5°C 55% of time

TABLE 9

McINDOE FALLS SLOW SAND FILTER PERFORMANCE
BACTERIA AND TURBIDITY REMOVAL

	Average Influent	Average Effluent	Average Removal
Ambient Raw Water Quality, June - September, 1982			
Std. Plate Count/1 mL	380	4.4	98.8%
Total Coliform/100 mL	1000	6	99.4%
Turbidity, NTU	1.2	0.3	75%
Ambient Raw Water Quality, October 1982 - January 1983			
Std. Plate Count/1 mL	130	2.4	98.1%
Total Coliform/100 mL	93	2	97.9%
Turbidity, NTU	1.1	0.1	91%
Raw Water Spiked with Sewage			
Std. Plate Count/1 mL (begin 9 day spike)	300	2.5	99.2%
Std. Plate Count/1 mL (end 9 day spike)	2200	150	93%
Total Coliform/100 mL (begin 9 day spike)	4500	60	98.7%
Total Coliform/100 mL (end 9 day spike)	16,000	2900	82%

progress at faster rates as temperature increases. Turbidity removal, however, was better during cold weather. This may be indicative of a different nature of turbidity-causing particles during different seasons. The latter portion of the October-January period would be very cold, with the marsh and pond water covered by ice. Cold weather and ice cover would reduce the level of activity by marsh-dwelling animals and minimize the influence of storms on water quality.

The sewage spiking study simulated a source water contamination problem. Bacteria removals during spiking were lower than removals during operation with ambient raw water quality.

Giardia cyst removal by the 13 gpm slow sand filter appears to have been influenced considerably by the operating temperture. The 99.99% removal at 21°C (Table 10) is similar to results obtained at 15°C at Colorado State University. The 0.5°C winter temperature was colder than any studied at CSU's Engineering Research Center, and cyst removal was also lower at 93.7%. This suggests that further work should be done with very cold water.

One DE filtration test was performed at McIndoe Falls to evaluate the 10 square foot filter's ability to remove Giardia cysts. Because previous studies (3, 4) and CSU results (14) had shown that DE filtration was effective for removal of cyst models and Giardia cysts, extensive research was not needed. The filtration test at McIndoe Falls resulted in 99.97% removal of Giardia cysts. The run was short, only 0.9 hour, in part because of the grade of diatomite used, Celite 503®. Use of a coarser grade would have given a longer run. The cyst removal obtained at a filtration flow of 59 L/min was very similar to results obtained in the 1 sq ft. pilot filter studies.

CONCLUSIONS

Large membrane filters with 5 um pores can be used to sample filtered waters for Giardia cysts.

Although the cyst recovery and counting procedures are not as precise as desired (± 30%), because cyst removal by water filtration processes often was observed to be on the order of 2, 3, or 4 log cycles, the excellent removal efficiencies observed were really the result of filter performance and were not caused by method variability.

Adequate chemical pretreatment of raw water must be accomplished if consistently high cyst removal is an operating goal for rapid rate granular media filtration.

Efficiency of cyst removal by coagulation-filtration is related to degree of turbidity removal and to filtered water turbidity.

Diatomaceous earth filters, when properly operated, can be depended upon to remove 99.9% or more of Giardia cysts from raw water.

TABLE 10

GIARDIA CYST REMOVAL AT McINDOE FALLS

Filtration Rate, m/hr	Temperature °C	Days Since Most Recent Filter Scraping	Number of 24 Hour Samples Collected	Cysts Recovered	Number of Samples With NO cysts	Cyst Removal
			Slow Sand Filter Results			
0.08	0.5°	34	5	4032	2	93.7%
0.08	21°	35	5	32	2	99.99%

Results of Diatomaceous Earth Filter Test

Filtration Rate, m/hr	Temperature °C	Cysts Applied	Actual Cysts Recovered	Fraction of Effluent Sampled	Cyst Removal
3.8	23°	8×10^6	48	5.3%	99.97%

Slow sand filters can remove 99.9% of applied cysts at a variety of operating rates. Condition of the filter bed influences filter efficiency.

Field scale research suggests that low temperatures may cause a decline in Giardia cyst removal by slow sand filters.

REFERENCES

1. Rendtorff, R. C. The Experimental Transmission of Giardia lamblia Among Volunteer Subjects. In Proceedings of Symposium on Waterborne Transmission of Giardiasis, Edited by W. Jakubowski and J. C. Hoff. EPA-600/9-79-01. p. 65. (June 1979).

2. Craun, G. F. and McCabe, L. J. Waterborne Disease Outbreaks in the United States and the Coliform Standard. In Proceedings of Workshop on Assessment of Microbiology and Turbidity Standards in Drinking Water, Edited by P. S. Berger and Y. Argaman. EPA 570-9-83-001. p. 9. (July 1983).

3. Logsdon, G. S., Symons, J. M., Hoye, R. L., Jr. and Arozarena, M. M. Alternative Methods for Removal of Giardia cysts and Cysts Models. Journal American Water Works Association. 73:2:111. (February, 1981)

4. DeWalle, F. B., Engeset, J., and Lawrence, J. Removal of Giardia lamblia Cysts from Drinking Water Plants. EPA Project Report, In Press.

5. Davies, R. B., Fukutaki, K., and Hibler, C. P. Cross Transmission of Giardia. EPA Report No. PB 83-1177478, Cincinnati, OH (January, 1983).

6. Hewlett, E. L., Andrews, J. S., Jr., Ruffier, J., and Schaefer, F. W. III. Experimental Infection of Mongrel Dogs with Giardia lamblia Cysts and Cultured Trophozoites. The Journal of Infectious Diseases. 145:1:89 (January 1982).

7. Bellamy, W. D., Al-Ani, M. Y., Silverman, G. P., Lange, K. P., Choi, S. I., Howell, D. G., Hibler, C. P., and Hendricks, D. W. Removal of Giardia lamblia from Water Supplies, Interim Report for EPA Cooperative Agreement CR808650-01, Environmental Engineering Program, Colorado State University, November 1982.

8. Silverman, G. P., Bellamy, W. D., and Hendricks, D. W. Slow Sand Filtration of Giardia lamblia Cysts and Other Substances, Phase I Report for EPA Cooperative Agreement CR8808650-02, Environmental Engineering Program, Colorado State University, September, 1983.

9. Standard Methods for the Examination of Water and Wastewater, 15th Ed. American Public Health Association, Washington, DC 1981.

10. Al-Ani, M. and Hendricks, D. W. Rapid Sand Filtration of Giardia Cysts. Presented at American Society of Civil Engineers National Conference on Environmental Engineering, Boulder, Colorado (July 6-8, 1983).

11. Bellamy, W. D., Silverman, G. P. and Hendricks, D. W. Giardia lamblia Removal by Slow Sand Filtration, in Proceedings of Sunday Seminar on Innovative Filtration Techniques, American Water Works Association Annual Conference, Las Vegas, Nevada (June 5, 1983).

12. Edzwald, J. K. Removal of Trihalomethane Precursors by Direct Filtration and Conventional Treatment. EPA Project Report and Project Summary, Cincinnati, Ohio. In Press (1983).

13. Hunter, J. V., Bell, G. R., and Henderson, C. N. Coliform Organism Removals by Diatomite Filtration. JAWWA, 58:9:1160 (Sept. 1966).

14. Lange, K. P., Bellamy, W. D., and Hendricks, D. W. Diatomaceous Earth Filtration -- Effects of Operating Conditions on the Removal of Giardia lamblia Cysts and Other Substances, Final Report for EPA Cooperative Agreement No. CR808650-02, Environmental Engineering Program, Colorado State University, October, 1983.